

Running Head: Progesterone buffers feeling of being excluded

Increased sensitivity to social exclusion during the luteal phase: Progesterone as
resilience factor buffering against ostracism?

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Abstract

A woman's social behaviour reportedly varies across the menstrual cycle. In this study, we estimated changes in sensitivity to social exclusion across the menstrual cycle and scrutinized the related role of progesterone. Forty-nine naturally cycling women played a virtual ball-tossing game (Cyberball) to manipulate social inclusion. All participants underwent inclusion and exclusion conditions during the late follicular and the luteal phase. We assessed salivary progesterone concentrations at each cycle phase. After each Cyberball session we measured positive/negative mood using the Multidimensional Mood State Questionnaire (MDMQ). Multilevel analyses indicated that women showed worse mood following exclusion as compared to inclusion conditions ($p=0.014$). Notably, this exclusion effect was more pronounced during the luteal phase than the late follicular phase ($p=0.029$). As expected, progesterone concentrations were higher during the luteal phase as compared to the late follicular phase, but interestingly, progesterone concentrations were negatively associated with exclusion effects. When accounting for mediation via progesterone, direct cycle-phase related differences in social exclusion effects even increased as compared to the model without mediator. These findings suggest that progesterone may function as buffer against negative feelings that result from being socially excluded. The relevance of these findings for Premenstrual Dysphoric Disorder (PMDD) are discussed, and we conclude that social exclusion may represent an important research domain criterion (RDoC) of relevance for PMDD, with progesterone pointing to new potential pharmacological targets.

KEYWORDS: Cyberball; late follicular phase; luteal phase; menstrual cycle; social exclusion; reproductive hormones

1 Introduction

The need to belong is a fundamental human motive and an essential requirement for security, reproductive success, and mental health (Baumeister and Leary, 1995; Smith et al., 1999). Yet, we all occasionally experience a brief episode of being ignored or excluded and even slight ostracism can be sufficient to cause pain and distress (e.g., Williams, 2007). Using an adaptationist framework, we estimated changes in sensitivity to social exclusion in naturally cycling women once during the late follicular and once during the luteal phase. We then scrutinized the related role of fluctuating estradiol, progesterone, and testosterone concentrations in order to explore physiological resilience factors underlying ostracism.

There is increasing evidence that naturally cycling women undergo a variety of psychological and behavioural changes throughout their menstrual cycle (Derntl et al., 2013; Goldstein et al., 2010; Wolohan et al., 2013). Evolutionary informed scholars have related these changes as serving to increase reproductive fitness: in the high-fertile late follicular phase preceding ovulation, psychological and behavioural changes should support the selection of genetically fit mates (Gangestad et al., 2007; Little et al., 2007) and should increase the chance for reproduction (Davis and Tran 2001; Krug et al., 2000). Conversely, in the postovulatory (luteal) phase, a woman's body is preparing for potential pregnancy (Müller and Hassel, 2012). During the luteal phase women should hence aim to reduce the risk of harm or disease (Fessler and Navarrete, 2003; Fleischman and Fessler, 2011). At the same time women should show increased affiliation motivation (Jones et al., 2008).

Behavioural changes during the luteal phase have often been linked to progesterone (e.g., Jones et al., 2008; Maner and Miller, 2014). Progesterone helps to secure pregnancy and its concentration increases substantially after ovulation.

Increased progesterone levels have been shown to be correlated with higher implicit affiliation motivation in men and women (Schultheiss et al., 2003; Wirth and Schultheiss, 2006). Moreover, high progesterone levels during the luteal phase have been associated with increased sensitivity for social information (Maner and Miller, 2014). Another study found that women showed increased progesterone levels after they experienced social exclusion (Seidel et al., 2013; but see Gaffey and Wirth, 2014; Radke et al., 2018). During the luteal phase, women often experience a recurrence of negative behavioral (e.g. fatigue), psychological (e.g. irritability) and physical symptoms (e.g. headaches) (Dickerson et al., 2003). Again, these negative symptoms have been associated with elevated progesterone levels (Smith et al., 2006).

The present study aims to investigate whether women are more sensitive to social exclusion during the luteal phase and if so, whether sensitivity to social exclusion can be explained by increased progesterone levels. One way to create situations of social exclusion in a laboratory setting is by using the so-called “Cyberball” game (Williams and Jarvis, 2006). Cyberball is a virtual ball-tossing game in which the participant is excluded from playing at one point. Being excluded during Cyberball results in lower levels of perceived belongingness, control, meaningful existence and self-esteem (Zadro et al., 2004). Furthermore, social exclusion leads to emotional responses such as jealousy (Harmon-Jones et al., 2009) and aggression (Chen et al., 2012).

The present study investigated the reactions to social exclusion across the menstrual cycle and in relation to the cyclic shifts in progesterone, estradiol and testosterone concentrations. While previous studies have reported hormonal reactions after being socially excluded (e.g., Radke et al., 2018; Seidel et al., 2013), the present study examined how levels of progesterone, estradiol and testosterone

as measured *before* social exclusion relate to mood changes experienced after being socially excluded. Specifically, we measured women's progesterone, estradiol and testosterone levels before playing the Cyberball game and assessed mood changes after experiencing social exclusion. Each woman was confronted with social exclusion (Cyberball) twice, once during the late follicular phase and once during the luteal phase. For menstrual cycle studies it is essential to accurately monitor the menstrual cycle, since cycle length can vary substantially between and within women (Jasienska, 2013; Lobmaier and Bachofner, 2018; Munster et al., 1992). We used multiple methods to maximize cycle monitoring accuracy. As a physiologically based fertility predictor we used OvaCUE© to estimate the peri-ovulatory phase. Peak fertility was then determined with urine tests measuring the luteinizing hormone and confirmed by the analysis of salivary estradiol, progesterone and testosterone concentrations.

We tested whether the experience of inclusion and exclusion while playing Cyberball varies across the menstrual cycle. We hypothesized that women show a stronger reduction in mood ratings following social exclusion during the luteal phase compared to the late follicular phase. To scrutinize the potential role of sex hormones on negative mood after social exclusion, we assessed the influence of progesterone, estradiol and testosterone concentrations on mood ratings after experiencing social inclusion and exclusion during the two menstrual cycle phases. Because affiliation motivation has been associated with progesterone, we expect progesterone levels to predict mood ratings after social exclusion.

2 Materials and methods

2.1 Participants

Of 86 women who initially showed interest in taking part in this study, datasets of 49 women were eventually included in the analyses (see flow chart Figure S1 in SI for an overview of the participants who dropped out and the reasons for non-participation at each stage). The included participants ranged in age between 18 and 33 years ($M = 24.30$ years; $SD = 3.91$ years). Twenty-nine were recruited from the general public via advertisements posted in public amenities and twenty were recruited from a pool of first-year psychology students. They received either course credits (psychology students) or 50 CHF (approximately 50 USD; participants from the general public) for their participation. All participants provided written informed consent to take part in this study and were treated in accordance with the ethical protocol approved by the Faculty of Human Sciences of the University of Bern and with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All women were selected on the basis of the following inclusion criteria: (a) between 18 and 35 years of age, (b) medication-free (including hormonal contraception for at least 3 previous months), (c) regular menstrual cycle (average length of between 25 and 35 days), (d) not pregnant or breastfeeding, and (e) no abortion in the previous six months. Participants indicated neither current nor previous history of psychiatric disorders or alcohol and drug abuse. Using the self-report PMS questionnaire (Ditzen et al., 2011) indicated that 14 (29.2%) participants reported premenstrual symptoms that have an impact on daily life. The presence of PMS symptoms was no exclusion criterion, as PMS symptoms are very common in the general population (between 50% and 80% of naturally cycling women; Dickerson et al., 2003; Ditzen et al., 2011; Halbreich et al., 2003).

2.2 Testing order

Participants were randomly assigned to two groups differing only in the order in which they were tested. Group 1 ($n = 25$) was first tested during the late follicular phase and then during the luteal phase, Group 2 ($n = 24$) was tested first during the luteal phase and then during the late follicular phase. This was done to control for potential effects of testing order. The groups did not significantly differ with respect to age ($t(47) = 0.059$; $p = 0.953$), PMS-ratings ($t(47) = 0.637$; $p = 0.527$), or trait mood ratings (PANAS positive: $t(47) = -0.854$; $p = 0.397$; PANAS negative: $t(47) = -0.766$; $p = 0.448$, as measured during the screening questionnaire, see Section 2.4, below).

2.3 Menstrual cycle monitoring

The menstrual cycle was monitored using various methods to ensure that women were tested at the right time. After agreeing to take part in this study, women were first interviewed via telephone in which we assessed the dates of the onsets of their last three menses. In cases where these data were not available, we assessed the self-reported cycle length and the approximate date of the onset of the actual menstrual cycle. To avoid unnecessary dropouts due to vacation, stressful life-events or a lack of time, we also asked participants to specify months in which study participation would work best for them. Participants were asked to report the onset of menstruation in the cycle in which they were planning to take part in the study.

Four days after menstruation onset participants started using an OvaCUE© fertility monitor (Fairhavenhealth, Bellingham, WA). The OvaCUE© is a hand-held electronic monitor with oral sensors detecting the electrical resistance of salivary secretion (<http://www.ovacue.com>). The electrical resistance of saliva changes with cyclical variations in oestrogen concentration (Fehring, 1996) and reaches a peak value five to seven days before ovulation; OvaCUE© can therefore be used as an

early ovulation predictor (Fehring, 1996). Participants conducted the OvaCUE© measurement daily and immediately after awakening. The assessment takes about three seconds and needs to be carried out for approximately 5 consecutive days, until the device can predict the date of peak fertility.

Two days before the date of predicted peak fertility women started to use urine tests measuring the luteinizing hormone (LH). We used one-step urine LH tests with a reported sensitivity of 10mIU/ml (David One Step Ovulation Tests, Runbio Biotech, China). Women were instructed to perform urine tests twice a day (morning and evening). After a positive test result participants continued the tests until the results became negative for two subsequent days. After positive testing, the women immediately reported to the laboratory and were then either tested within 48 hours of LH surge and then again 7 days later (late follicular-luteal group) or they were scheduled 7 days after the measured peak of the LH surge (luteal-late follicular group). Participants assigned to the luteal-late follicular group again assessed the LH surge in the following cycle and were then tested within 48 hours of the next LH peak.

On the days of testing, women additionally provided saliva samples from which we assessed levels of estradiol, testosterone and progesterone. Fifteen minutes after their arrival in the laboratory, participants were asked to collect approximately 7.5 ml of saliva in plastic tubes (Salicaps, IBL International GmbH, Hamburg, Germany). To control for potential factors that are known to influence hormone assessments from saliva, we asked the participants to avoid excessive physical activity and drinking alcohol and to refrain from using drugs on the days of LH testing and 12 hours prior to the scheduled session. Participants were further instructed to refrain from eating and to abstain from caffeine and smoking for at least 1h prior each experimental session. Participants were asked to rinse their mouth with fresh water and to wait

approximately 5 min before providing saliva. The plastic tubes were closed and stored at -20 °C until the salivary samples were analysed for concentrations of estrogen, progesterone and testosterone by an independent laboratory (Dresden Lab Service GmbH, Dresden, Germany) using commercially available radioimmunoassay kits adopted for the analysis of salivary samples (IBL International, Hamburg, Germany). Inter-assay coefficients were below 12% and intra-assay coefficients were below 10%.

2.4 Task and procedure

As soon as participants reported the onset of menstruation, we asked them to complete an online survey (EFS Survey, Questback, Berlin, Germany) with which we collected demographic data such as age, sexual orientation, whether they have any children, whether they currently are in a romantic relationship, and if yes, how long they have been in this relationship. We further assessed subjective ratings of premenstrual symptoms (PMS) using the German PMS questionnaire (Ditzen et al., 2011) and the Positive and Negative Affect Scale (PANAS; Watson et al., 1988). In the PANAS, women were asked to rate how their mood was during the last year (trait).

All test session took place in a laboratory at the University of Bern. To control for circadian hormone variability, all participants were tested between 11am and 6pm, and both test sessions were scheduled to take place at the same time of day. This time window was chosen because hormone levels show less variation in the afternoon than in the early morning (cf., Caufriez et al., 2009)

The lab-testings lasted approximately 30 minutes each. Upon arrival at the laboratory, participants gave their written informed consent and provided the salivary

sample. We applied a modified version of the Cyberball task developed by (Williams and Jarvis, 2006). Participants were informed that they would play a virtual ball tossing game via Internet with two other players. Participants were informed that these players were real players seated in separate rooms. In reality, these players were computer generated. Participants were told that the study examines the effects of mental visualization and that their task was to create a vivid mental image of the game scenery (Williams and Jarvis, 2006; see Figure S2 for a screenshot of the Cyberball scenario). Participants viewed pictures of the players presented on the right (Player 1) and the left side of the screen (Player 3). The picture of Player 1 always showed a man, the picture of Player 3 always was a woman. Different pictures were used in the first and second testing, and all pictures were “medium” attractive as rated in a pre-test. The Cyberball paradigm was presented on a tablet computer (Samsung Galaxy Note, 2.0) and was played online using Google Chrome Explorer (Chrome 35). In each session, participant played three rounds. Each round consisted of thirty ball tosses and took about two minutes to complete. When participants received the ball, they had to choose to whom they wanted to throw the ball by clicking on the respective player’s picture using a standard computer mouse. An algorithm controlled the behaviour of the computer-generated players. In the first round, the inclusion round, the participant received 10 out of 30 balls played. In the following exclusion round, the participant received only 4 out of 30 balls played. The rest of the balls were played between the computer-generated players. The last round was an inclusion round and was carried out for ethical reasons. After the first inclusion and the subsequent exclusion round participants completed a questionnaire. The first part of this questionnaire was used to support the cover story and consisted of few questions about the participants’ mental images during the game. The second part assessed the experience of the Cyberball manipulation. First

participants had to estimate the percentage of balls they received during the round. Then participants answered an abbreviated version of the Need-Threat Questionnaire (NTQ, Williams, 2009) to check whether the exclusion manipulation worked. We included only one question per dimension to ensure that any exclusion induced mood change would still be observable in the mood assessment. Specifically, we included the items “I had the feeling that I belonged to the group during the game” (Belongingness), “I had the feeling that I could influence the direction of the game” (Control), “I was concerned about what the other players thought about me during the game” (Self-Esteem), “I had the feeling that my presence during the game was important” (Meaningful Existence). Mood was assessed using the short form of the German multidimensional-mood-state questionnaire (Mehrdimensionaler Befindlichkeitsfragebogen “MDBF”; Steyer et al., 1994). The MDBF assesses mood state on three dimensions (*good-bad*, *awake-tired*, *calm-nervous*) and has been shown to be a time-efficient and reliable instrument for assessing mood in clinical and experimental settings (Heinrichs and Nater, 2002). Questionnaire items were presented on a 15.4-inch laptop monitor (HP Pavilion dv6, Windows 7, 64-bit) using internet explorer (V.11) and unipark software (Questback, Berlin, Germany). Both test sessions followed the exact same procedure except that after the second session participants were fully debriefed.

Before debriefing the participants, we checked whether they knew the paradigm or guessed the aim of the study. One participant knew the paradigm and was hence excluded from the analyses (see Flowchart in supplemental online material). The rest were naïve to the purpose of the study and did not know the paradigm. Participants were told not to talk about this study to their friends and colleagues, so that other participants remained naïve.

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279 **3 Statistical analyses**

280 We checked the data for distribution properties and verified normality by
 281 inspecting histograms and qq-plots. We log-transformed hormone concentration
 282 values to approximate normal distribution. For descriptive analyses, we calculated
 283 means and standard deviations for continuous normally distributed variables and
 284 absolute and relative frequencies for the categorical variable with categories outlined
 285 in Table S1.

286 To confirm that the two Cyberball conditions ‘inclusion’ vs. ‘exclusion’ induced
 287 the expected contrast, we conducted a manipulation check. To do so, we entered
 288 each scale of the Need Threat Questionnaire (NTQ) as outcome in separate linear
 289 mixed-effects models (Singer and Willett, 2003), to estimate changes in ‘belonging’,
 290 ‘self-esteem’, ‘meaningful existence’, and ‘control’ between the inclusion and
 291 exclusion condition by adding ‘ostracism’ as predictor. We added the factors ‘cycle
 292 phase’ (luteal phase vs. late follicular phase) and ‘assessment sequence’ (luteal
 293 phase assessed first vs. late follicular phase assessed first) as covariates.

294 Next, to test our main hypotheses that ostracism effects (inclusion vs.
 295 exclusion) on mood are more pronounced during the luteal phase as compared to the
 296 late follicular phase, we entered each scale of the MDMQ as outcome variable in
 297 separate linear mixed-effects models (Singer and Willett, 2003). We first estimated
 298 the effects of the factors ‘ostracism’ (inclusion vs. exclusion) and ‘cycle phase’ (luteal
 299 phase vs. late follicular phase) by only including these two categorical predictors. We
 300 then repeated the analyses after adding the interaction effect of ‘ostracism’ and ‘cycle
 301 phase’ as additional predictor. In all these analyses, we added the factor ‘assessment

sequence' (luteal phase assessed first vs. late follicular phase assessed first) as covariate.

To scrutinize changes in salivary concentrations of the hormones progesterone, estradiol, and testosterone between cycle phases we entered each hormone concentration as outcome in separate linear mixed-effects models (Singer and Willett, 2003), and estimated changes in the hormone concentrations between the luteal phase and the late follicular phase by including 'cycle phase' as predictor. We added the factor 'assessment sequence' (luteal phase assessed first vs. late follicular phase assessed first) as covariate.

For all linear mixed-effects models we used an unstructured covariance matrix to account for the time dependence among repeated measures of the within-subjects factors 'ostracism' and 'cycle phase'.

Finally, we estimated whether the associations between cycle phase and ostracism related differences in mood were mediated by progesterone concentrations (see Figure 1). Therefore, for each scale of the MDMQ, we conducted separate multilevel structural equation models (Preacher et al., 2010), with the predictor 'cycle phase', the outcome 'ostracism-related mood differences' (i.e. difference in mood between the inclusion and the exclusion condition), and the mediator 'progesterone concentration', assuming random intercepts and fixed slopes.

Linear mixed-effects models and multilevel structural equation models accommodated missing data. All tests were two-tailed, we set the significance level at 0.05 and calculated 95% confidence intervals (CI) where appropriate. We used the statistical software package Mplus for Mac (version 6.12) for the multilevel structural

equation models, and IBM SPSS Statistics for Mac (Version 21) for all other data analyses.

----Figure 1 about here----

4 Results

Analyses are based on data of 49 women (see Table S1 in SI for characteristics of the study sample).

4.1 Manipulation check

We employed the Need Threat Questionnaire (NTQ, Williams, 2009) to check whether our inclusion/exclusion manipulation worked. In the inclusion condition of the Cyberball paradigm, women reported higher levels of ‘belonging’, ‘meaningful existence’, and ‘control’ than in the exclusion condition, confirming successful induction of subjective ostracism by the Cyberball paradigm. There was no statistically significant difference in ‘self-esteem’. Respective results are depicted in Table 1.

----Table 1 about here----

4.2 Differences in mood related to ostracism and cycle phase

Linear mixed-effects models revealed an ostracism effect for MDMQ mood dimensions ‘good-bad’ and ‘awake-tired’, but not ‘calm-nervous’, with women reporting worse mood (*Estimate*=0.224, *standard error (SE)*=0.088, 95%CI [0.047, 0.401], *t*=2.544, *df*=49, *p*=0.014) and being more tired (*Estimate*=0.276, *SE*= 0.071,

95%CI [0.133, 0.418], $t=3.908$, $df=49$, $p<0.001$) in the exclusion as compared to the inclusion condition of the Cyberball paradigm. There was a significant interaction effect between ostracism and cycle phase for good-bad mood ($Estimate=0.332$, $SE=0.147$, 95%CI [0.036, 0.628], $t=2.255$, $df=49$, $p=0.029$), indicating a stronger ostracism effect in the luteal phase as compared to the late follicular phase (see Figure 2, Table 2).

----Figure 2 about here----

----Table 2 about here----

4.3 Differences in hormone concentrations between cycle phases and mediation of the association between cycle phase and mood via progesterone concentrations

Linear mixed-effect models revealed that salivary progesterone and estradiol concentrations, but not testosterone concentrations were higher during the luteal phase as compared to the late follicular phase (see Table 3).

----Table 3 about here----

Results from multilevel structural equation models related to the MDMQ scale 'good-bad mood' are depicted in Table 4, and results related to the MDMQ scales 'awake-tired' and 'calm-nervous' are depicted in supplemental material Table S2, Table S3, Figure S3 and Figure S4, respectively. Please refer to Figure 1 for the outline of the mediation analyses. As expected and in line with the result from the linear mixed-effect model outlined above (see Table 3), progesterone concentrations were higher during the luteal phase than during the follicular phase (mediation path

a). Notably, higher progesterone levels were associated with smaller ostracism effects (mediation path b) with regard to the mood dimensions 'good-bad', 'awake-tired', and 'calm-nervous', when adjusting for cycle phase. This led to 'inconsistent mediation' effects (MacKinnon et al., 2007), in the way that in all three models, the indirect/mediated effects and the direct effects were of opposite sign (negative vs. positive).

----Table 4 about here----

Hence, with regard to 'good-bad mood' there was a statistically significant mediation effect of progesterone concentration ($a*b$; *Estimate*=-0.418, *SE*=0.145, 95%CI [-0.656, -0.180], $p=0.004$), resulting in an even stronger direct effect (c' ; *Estimate*=0.772, *SE*= 0.225, 95%CI [0.401, 1.143], $p=0.001$) than the total effect (c ; *Estimate*=0.354, *SE*=0.148, 95%CI [0.110,0.598], $p=0.017$).

With regard to ostracism-related differences in mood dimensions 'awake-tired' and 'calm-nervous' there were no statistically significant total effects (c) of cycle phase. This is in line with the results from the above-reported linear mixed-effect models (Table 2) indicating no statistically significant interaction between ostracism and cycle phase for the outcomes 'awake-tired' and 'calm-nervous'. However, due to the statistically significant and inconsistent mediation effects of progesterone ($a*b$), mediation analyses revealed statistically significant direct effects (c') of cycle phase on ostracism-related differences in the dimensions 'awake-tired' (*Estimate*=0.340, *SE*= 0.170, 95%CI [0.060, 0.619], $p=0.046$) and 'calm-nervous' (*Estimate*=0.388, *SE*= 0.187, 95%CI [0.081, 0.695], $p=0.037$) (see Table S2 and Table S3, respectively).

5 Discussion

The main goals of this study were to estimate the changes in sensitivity to social exclusion (ostracism) in different cycle phases and to scrutinize the related role of fluctuating progesterone concentrations across the menstrual cycle in naturally cycling women. We found that during the more vulnerable luteal phase women were more sensitive to rejection than during the late follicular phase. At the same time sensitivity to rejection was associated with lower progesterone levels. This finding suggests that higher progesterone concentrations buffer against feelings of rejection.

Women in the luteal phase are potentially pregnant. Because pregnancy calls for increased need for social support, an evolutionary informed interpretation of these findings is that social exclusion may represent a higher threat during the luteal phase and may therefore result in more negative mood than during the follicular phase. In this adaptationist framework, progesterone, which coincidentally is raised during the luteal phase, may function as a resilience factor buffering against the negative feelings experienced after being ostracized.

During the luteal phase, naturally cycling women often experience a drop in mood (Dickerson et al., 2003). While most premenopausal women experience some level of negative premenstrual symptoms (Dickerson et al., 2003), up to 8% suffer to such a degree that it interferes with normal functioning (Premenstrual Dysphoric Disorder, PMDD; Bhatia and Bhatia, 2002; Wittchen et al., 2002). Negative mood is one of the most prominent symptoms of PMDD and such dips in mood have been related to progesterone levels which increase during the luteal phase and decrease rapidly at the onset of menses (e.g., Smith et al., 2006). It is striking that the social components of PMDD have received much less attention, despite the fact that the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) explicitly

mentions marked affective lability such as increased sensitivity to rejection as well as increased interpersonal conflicts as symptoms of PMDD. From a clinical and research domain criteria (RDoC) perspective, our findings point towards reduced progesterone levels and/or related neurotransmission as a potential target to buffer against suffering related to the symptom dimension ‘sensitivity to social rejection’ that is of relevance for PMDD and beyond. This converges with recent data suggesting that neurotransmission related to allopregnanolone, a metabolite from progesterone, is a promising target to treat PMDD (Bixo et al., 2017; Martinez et al., 2016). Unfortunately, as our sample includes only 14 women with impairing premenstrual symptoms, it is too small to conduct meaningful statistical analyses in this subgroup. Hence, future studies with a clinical sample should scrutinize, whether our main findings can also be detected in women with PMDD.

The present results substantiate findings of previous work in which social exclusion in Cyberball resulted in more negative mood (e.g., Seidel et al. 2013; Williams and Jarvis, 2006). Furthermore, the results are in line with previous findings suggesting an increased sensitivity for social information during the luteal phase (Maner and Miller, 2014). But while existing literature often argues that this increased sensitivity results from the heightened progesterone concentrations during the luteal phase, we found that progesterone was related to reduced negative mood after experiencing social exclusion. Specifically, in the luteal phase, high progesterone values were associated with less negative mood ratings after experiencing social exclusion. In the late follicular phase we found no relation between progesterone concentrations and mood ratings. Even though we found that during the luteal phase, which is characterized by increased progesterone levels, women reacted more sensitively to social exclusion, our data do not support the view that elevated

progesterone levels are responsible for this increased sensitivity to ostracism. Rather, the present findings support the biphasic action model of progesterone metabolites on mood (Andreen et al., 2009). According to this model low concentrations of allopregnanolone increase negative mood changes via GABA_A systems, while high concentrations have calming effects. Further studies are needed to verify the role of luteal progesterone in social behaviour.

In the present study, the order of inclusion/exclusion blocks in the Cyberball game was held constant, that is, participants always experienced inclusion before being excluded (see also Masten et al., 2011; Radke et al., 2018). We hence cannot fully rule out that the worse mood and increased tiredness after exclusion vs. inclusion might be confounded with the temporal course of the experiment. We note however, that our most important finding was that worse mood after exclusion was modulated by cycle phase. It is hence unlikely that using this commonly adopted sequence of inclusion followed by exclusion in a constant order detracts from our main finding, namely that worse mood related to the exclusion condition was more pronounced during the luteal phase, compared to the follicular phase. We note that we did not assess mood after the last round (inclusion condition), since our main outcome variable was mood changes after exclusion. The last round (inclusion condition) was merely included for ethical reasons: Because we could not debrief the participants after the first session, we needed to let them finish the session with the feeling of being included.

The need to belong is a fundamental human motive and feeling left out causes distress. Here we provide evidence that naturally cycling women are more sensitive to social exclusion during the luteal phase as compared to the late follicular phase. Further, high progesterone levels – which characterize the luteal phase – were

negatively related to feelings of being left out. During the luteal phase women often experience a dip in mood, which in some cases can lead to Premenstrual Dysphoric Disorder (PMDD). Our findings provide evidence that progesterone acts as resilience factor, buffering against negative feelings that result from being socially excluded, thereby pointing to new potential pharmacological targets to treat PMDD and other premenstrual symptoms.

Author contribution: JSL, FP, VL and GM designed the study, VL, FP and JSL collected the data, AHM and GM analysed the data, JSL, VL, AHM and GM wrote the manuscript. All authors approved the final version of the manuscript.

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493 **References**

- 494 Andreen, L., Nyberg, S., Turkmen, S., van Wingen, G., Fernandez, G., Backstrom, T., 2009. Sex steroid
495 induced negative mood may be explained by the paradoxical effect mediated by GABAA modulators.
496 *Psychoneuroendocrinology* 34, 1121-1132.
- 497 Baumeister, R.F., Leary, M.R., 1995. The need to belong: desire for interpersonal attachments as a
498 fundamental human motivation. *Psychol Bull* 117, 497-529.
- 499 Bhatia, S.C., Bhatia, S.K., 2002. Diagnosis and treatment of premenstrual dysphoric disorder. *Am Fam*
500 *Physician* 66, 1239-1248.
- 501 Bixo, M., Ekberg, K., Poromaa, I.S., Hirschberg, A.L., Jonasson, A.F., Andreen, L., Timby, E., Wulff, M.,
502 Ehrenborg, A., Backstrom, T., 2017. Treatment of premenstrual dysphoric disorder with the GABAA
503 receptor modulating steroid antagonist Sepranolone (UC1010)-A randomized controlled trial.
504 *Psychoneuroendocrinology* 80, 46-55.
- 505 Caufriez, A., Leproult, R., L'Hermite-Baleriaux, M., Moreno-Reyes, R., Copinschi, G., 2009. A potential
506 role of endogenous progesterone in modulation of GH, prolactin and thyrotrophin secretion during
507 normal menstrual cycle. *Clin. Endocrinol.* 71, 535-542.
- 508 Chen, Z.S., DeWall, C.N., Poon, K.T., Chen, E.W., 2012. When destiny hurts: Implicit theories of
509 relationships moderate aggressive responses to ostracism. *J. Exp. Soc. Psychol.* 48, 1029-1036.
- 510 Davis, S.R., Tran, J., 2001. Testosterone influences libido and well being in women. *Trends Endocrinol*
511 *Metab* 12, 33-37.
- 512 Derntl, B., Hack, R.L., Kryspin-Exner, I., Habel, U., 2013. Association of menstrual cycle phase with the
513 core components of empathy. *Horm Behav* 63, 97-104.
- 514 Dickerson, L.M., Mazyck, P.J., Hunter, M.H., 2003. Premenstrual syndrome. *Am Fam Physician* 67,
515 1743-1752.
- 516 Ditzen, B., Nussbeck, F., Drobnjak, S., Spörri, C., Wüest, D., Ehlert, U., 2011. Validierung eines
517 deutschsprachigen DSM-IV-TR basierten Fragebogens zum prämenstruellen Syndrom. *Zeitschrift für*
518 *klinische Psychologie und Psychotherapie* 40, 149–159.
- 519 Fehring, R.J., 1996. A comparison of the ovulation method with the CUE ovulation predictor in
520 determining the fertile period. *J Am Acad Nurse Pract* 8, 461-466.
- 521 Fessler, D.M., Navarrete, C.D., 2003. Domain-specific variation in disgust sensitivity across the
522 menstrual cycle. *Evol Hum Behav* 24, 406-417.
- 523 Fleischman, D.S., Fessler, D.M., 2011. Progesterone's effects on the psychology of disease avoidance:
524 support for the compensatory behavioral prophylaxis hypothesis. *Horm Behav* 59, 271-275.
- 525 Gaffey, A.E., Wirth, M.M., 2014. Stress, rejection, and hormones: Cortisol and progesterone reactivity
526 to laboratory speech and rejection tasks in women and men. *F1000Res* 3, 208.
- 527 Gangestad, S.W., Garver-Apgar, C.E., Simpson, J.A., Cousins, A.J., 2007. Changes in women's mate
528 preferences across the ovulatory cycle. *J Pers Soc Psychol* 92, 151-163.
- 529 Goldstein, J.M., Jerram, M., Abbs, B., Whitfield-Gabrieli, S., Makris, N., 2010. Sex differences in stress
530 response circuitry activation dependent on female hormonal cycle. *J Neurosci* 30, 431-438.
- 531 Halbreich, U., Borenstein, J., Pearlstein, T., Kahn, L.S., 2003. The prevalence, impairment, impact, and
532 burden of premenstrual dysphoric disorder (PMS/PMDD). *Psychoneuroendocrinology* 28 Suppl 3, 1-
533 23.
- 534 Harmon-Jones, E., Peterson, C.K., Harris, C.R., 2009. Jealousy: novel methods and neural correlates.
535 *Emotion* 9, 113-117.
- 536 Heinrichs, M., Nater, U., 2002. The Mehrdimensionale Befindlichkeitsfragebogen. *Zeitschrift Fur*
537 *Klinische Psychologie Und Psychotherapie* 31, 66-67.
- 538 Jasienska, G., 2013. The fragile wisdom: an evolutionary view on women's biology and health.
539 Harvard University Press, Cambridge MA.
- 540 Jones, B.C., DeBruine, L.M., Perrett, D.I., Little, A.C., Feinberg, D.R., Law Smith, M.J., 2008. Effects of
541 menstrual cycle phase on face preferences. *Arch Sex Behav* 37, 78-84.

- Krug, R., Plihal, W., Fehm, H.L., Born, J., 2000. Selective influence of the menstrual cycle on perception of stimuli with reproductive significance: an event-related potential study. *Psychophysiology* 37, 111-122.
- Little, A.C., Jones, B.C., Burriss, R.P., 2007. Preferences for masculinity in male bodies change across the menstrual cycle. *Horm Behav* 51, 633-639.
- Lobmaier, J.S., Bachofner, L.M., 2018. Timing is crucial: Some critical thoughts on using LH tests to determine women's current fertility. *Horm Behav*.
- MacKinnon, D.P., Fairchild, A.J., Fritz, M.S., 2007. Mediation analysis. *Annu Rev Psychol* 58, 593-614.
- Maner, J.K., Miller, S.L., 2014. Hormones and social monitoring: Menstrual cycle shifts in progesterone underlie women's attention to signs of social support. *Evol Hum Behav* 35, 9-16.
- Martinez, P.E., Rubinow, D.R., Nieman, L.K., Koziol, D.E., Morrow, A.L., Schiller, C.E., Cintron, D., Thompson, K.D., Khine, K.K., Schmidt, P.J., 2016. 5alpha-Reductase Inhibition Prevents the Luteal Phase Increase in Plasma Allopregnanolone Levels and Mitigates Symptoms in Women with Premenstrual Dysphoric Disorder. *Neuropsychopharmacology* 41, 1093-1102.
- Masten, C.L., Colich, N.L., Rudie, J.D., Bookheimer, S.Y., Eisenberger, N.I., Dapretto, M., 2011. An fMRI investigation of responses to peer rejection in adolescents with autism spectrum disorders. *Dev Cogn Neurosci* 1, 260-270.
- Müller, W., Hassel, M., 2012. *Entwicklungsbiologie und Reproduktionsbiologie des Menschen und bedeutender Modellorganismen*, 5th ed. ed. Springer, Berlin.
- Munster, K., Schmidt, L., Helm, P., 1992. Length and variation in the menstrual cycle--a cross-sectional study from a Danish county. *Br J Obstet Gynaecol* 99, 422-429.
- Preacher, K.J., Zyphur, M.J., Zhang, Z., 2010. A general multilevel SEM framework for assessing multilevel mediation. *Psychol Methods* 15, 209-233.
- Radke, S., Seidel, E.M., Boubela, R.N., Thaler, H., Metzler, H., Kryspin-Exner, I., Moser, E., Habel, U., Derntl, B., 2018. Immediate and delayed neuroendocrine responses to social exclusion in males and females. *Psychoneuroendocrinology* 93, 56-64.
- Schultheiss, O.C., Dargel, A., Rohde, W., 2003. Implicit motives and gonadal steroid hormones: effects of menstrual cycle phase, oral contraceptive use, and relationship status. *Horm Behav* 43, 293-301.
- Seidel, E.M., Silani, G., Metzler, H., Thaler, H., Lamm, C., Gur, R.C., Kryspin-Exner, I., Habel, U., Derntl, B., 2013. The impact of social exclusion vs. inclusion on subjective and hormonal reactions in females and males. *Psychoneuroendocrinology* 38, 2925-2932.
- Singer, J.D., Willett, J.B., 2003. *Applied Longitudinal Data Analysis*. Oxford University Press, Oxford.
- Smith, E.R., Murphy, J., Coats, S., 1999. Attachment to groups: theory and measurement. *J Pers Soc Psychol* 77, 94-110.
- Smith, S.S., Ruderman, Y., Frye, C., Homanics, G., Yuan, M., 2006. Steroid withdrawal in the mouse results in anxiogenic effects of 3alpha,5beta-THP: a possible model of premenstrual dysphoric disorder. *Psychopharmacology (Berl)* 186, 323-333.
- Steyer, R., Schwenkmezger, P., Notz, P., Eid, M., 1994. Testtheoretische Analysen des mehrdimensionalen Befindlichkeitsfragebogen (MDBF). *Diagnostica* 40, 320-328.
- Watson, D., Clark, L.A., Tellegen, A., 1988. DEVELOPMENT AND VALIDATION OF BRIEF MEASURES OF POSITIVE AND NEGATIVE AFFECT - THE PANAS SCALES. *Journal of Personality and Social Psychology* 54, 1063-1070.
- Williams, A.M., 2007. Ostracism: The kiss of social death. *Social and Personality Psychology Compass* 1, 236-247.
- Williams, K.D., 2009. Ostracism: A Temporal Need-Threat Model. *Adv Exp Soc Psychol* 41, 275-314.
- Williams, K.D., Jarvis, B., 2006. Cyberball: a program for use in research on interpersonal ostracism and acceptance. *Behav Res Methods* 38, 174-180.
- Wirth, M.M., Schultheiss, O.C., 2006. Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol. *Horm Behav* 50, 786-795.
- Wittchen, H.U., Becker, E., Lieb, R., Krause, P., 2002. Prevalence, incidence and stability of premenstrual dysphoric disorder in the community. *Psychol Med* 32, 119-132.

593 Wolohan, F.D., Bennett, S.J., Crawford, T.J., 2013. Females and attention to eye gaze: effects of the
594 menstrual cycle. *Exp Brain Res* 227, 379-386.
595 Zadro, L., Williams, K.D., Richardson, R., 2004. How low can you go? Ostracism by a computer is
596 sufficient to lower self-reported levels of belonging, control, self-esteem, and meaningful existence.
597 *J. Exp. Soc. Psychol.* 40, 560-567.

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Figure Legends

Figure 1. (A) Illustration of a total effect c of cycle phase on ostracism-related mood differences. (B) Illustration of a mediation design, in which cycle phase is supposed to exert an indirect effect ($a*b$) on ostracism-related mood differences through progesterone concentrations (a = effect of cycle phase on progesterone concentrations; b = effect of progesterone concentrations on ostracism-related mood differences) and the direct effect of cycle phase on ostracism-related mood differences c' ($c' = c - a*b$).

Figure 2. Good-bad mood assessed using the Multidimensional Mood Questionnaire (MDMQ); SEM, standard error of mean.